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A comparative study of the effects of ketotifen, disodium cromoglycate, and beclomethasone dipropionate on bronchial mucosa and asthma symptoms in patients with atopic asthma

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Asthma is a chronic inflammatory disorder of the airways that is characterized by infiltration of many inflammatory cells into the bronchial mucosa. We compared the effects of ketotifen, disodium cromoglycate (DSCG), and beclomethasone dipropionate (BDP) on inflammatory cells in the bronchial mucosa and on the asthma symptoms of patients with atopic asthma.

In this 12-week parallel study, 32 patients were randomly allocated to either the ketotifen group (2 mg day^{-1}, n=13), DSCG group (8 mg day^{-1}, n=9) or BDP (400 μg day^{-1}, n=10). Each subject recorded daily asthma symptoms and peak expiratory flow (PEF). Before and after treatment, pulmonary function and bronchial responsiveness to methacholine were evaluated, and fibreoptic bronchoscopy and biopsy were performed before and after treatment. Biopsy specimens were obtained by bronchoscopy. We performed immunohistochemistry using specific monoclonal antibodies for activated eosinophils (EG2), mast cells (AA1), and T cells (CD3, CD4, and CD8).

Our clinical findings showed significant improvement in symptom score and bronchial responsiveness (P<0·01 each) in all groups. Both the DSCG and the BDP groups had significantly better symptom scores than the ketotifen group (P<0·05, both groups). PEF significantly increased in the DSCG group in comparison to the ketotifen (P<0·01) and BDP (P<0·05) groups. FEV_1% increased significantly in the DSCG (P<0·01) and BDP (P<0·05) groups in comparison to the ketotifen group. Compared with their baseline values, treatment significantly decreased $EG2^+$ activated eosinophils, and $CD3^+$ and $CD4^+$ T cells, in each group (P<0·01). Both the DSCG (P<0·05) and the BDP groups (P<0·01) exhibited significant decreases in $AA1^+$ mast cell count, but this was not observed in the ketotifen group. Comparing before- and after-treatment values, only the DSCG group exhibited a significant decrease in the number of $CD8^+$ T cells (P<0·01).

Ketotifen, DSCG, and BDP all showed anti-inflammatory activity as determined by examination of the bronchial mucosa of asthmatic patients; and both the DSCG and BDP groups had better clinical responses than the ketotifen group.

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Introduction

Bronchial asthma is a chronic inflammatory disorder of the airways in which the bronchial mucosa is infiltrated by many types of inflammatory cells, including eosinophils, mast cells, and T lymphocytes (1). Descriptions of the pathological features of bronchial asthma are largely based on the findings of autopsy studies (2). More recently, bronchial biopsies (3,4) and bronchoalveolar lavage (BAL) (5) obtained from patients with asthma demonstrated the presence of local inflammation by inflammatory cells even in a mild disease. In the prevention and management of asthma, increased emphasis has been placed on anti-inflammatory drugs (1). Corticosteroids, disodium cromoglycate (DSCG), nedocromil sodium, sustained-release theophylline, long-acting β_2-agonists, and oral antiallergic compounds are drugs, which are used to 'control' symptoms. The use of antihistamines in treatment of asthma has not yet been established. Ketotifen is one representative of the anti-allergic compounds. Oral anti-allergic compounds are frequently used as anti-asthma agents in Japan. Ketotifen has antihistaminic and anti-allergic actions: it can inhibit the release of chemical mediators from mast cells (6),

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TABLE 1. Subject characteristics

	Ketotifen (n=13)	DSCG (n=9)	BDP (n=10)
Age (years)	27 (16–50)	26 (20–31)	30 (23–44)
Sex (M:F)	7:6	6:3	7:3
Symptom score	3·5 (1·5–10·5)	3·7 (1·0–6·4)	5·0 (1·1–12·8)
PEF ($l\ min^{-1}$)	450 (320–580)	350 (300–540)	425 (250–550)
FEV_1% pred.	72·0 (58·7–89·9)	63·2 (57·6–90·5)	66·0 (59·4–74·9)
D_{min} (unit)	0·32 (0·11–2·24)	0·34 (0·15–2·56)	0·68 (0·21–2·03)
IgE ($IU\ ml^{-1}$)	1045·0 (276–3430)	500·0 (243–925)	749·9 (171–1953)

PEF, peak expiratory flow; D_{min}, dose of methacholine as a measure of bronchial sensitivity. Unit: equal to 1 min of 1·0 $mg\ ml^{-1}$ aerosol inhalation of methacholine.

and in patients with asthma it can inhibit the release of histamine and leukotrienes from basophils (7). Clinical studies done in Europe have shown that prophylactic treatment with ketotifen can be effective in patients with asthma (8,9). Clinical studies have shown ketotifen is effective in treatment of asthma when compared with placebo (10), theophylline (11), and DSCG (12); and ketotifen is shown to have dose-reducing effects on steroids (13,14). Though anti-inflammatory effects of inhaled corticosteroids have already been proven (15–17), these effects have not been examined for ketotifen and DSCG.

This study was therefore undertaken to compare the effects of ketotifen, DSCG, and beclomethasone dipropionate (BDP) on bronchial mucosa, asthma symptoms, and bronchial responsiveness, in patients with asthma.

Methods

SUBJECTS

Thirty-two asthmatic patients were recruited from our hospital. Asthma was diagnosed based on the clinical history of intermittent chest tightness, wheezing, coughing, or shortness of breath and documented reversible airflow obstruction [20% improvement in peak expiratory flow (PEF) or forced expiratory volume in 1 s (FEV_1), either spontaneously or in response to inhaled β_2-agonists]. All patients had mild to moderate atopic asthma, which was diagnosed according to two or more positive skin prick test results and the presence of aeroallergen-specific IgE. No patients had received inhaled or oral corticosteroids, or any other anti-inflammatory drugs such as sodium cromoglycate, or nedocromil sodium, in the preceding 4 months. The patients used β_2-agonists only when necessary. Patients who smoked or whose FEV_1 was less than 50% of the predicted value were excluded. Patients showed no signs of upper or lower respiratory disease for at least 2 weeks before entering the study. The Ethics Committee of Toho University School of Medicine approved the study protocol, and all subjects gave informed written consent.

STUDY DESIGN

At the initial visit, the patients were examined and a full disease history was obtained. Skin prick tests to common aeroallergens (house-dust mite, cat fur, dog fur and grass pollen) were performed and blood was taken for total and specific IgE (Phadebas RAST; Pharmacia, Uppsala, Sweden). The patients measured their PEF in the morning and evening using a personal best peak flowmeter (Health Scan, Cedar Grove, NJ, U.S.A.), and recorded their results on a diary card. After 6 days, patients were readmitted after having abstained from inhaled β_2-agonists for at least 12 h. Spirometry was performed and methacholine inhalation test measurements were taken according to the method of Takishima *et al.* (18). Briefly, using an 'Astograph' (TCK-6000CV, Chest MI Co., Tokyo, Japan), the dose–response curves of respiratory resistance were determined. Baseline values were measured after the inhalation of an isotonic saline solution, after which increasing concentrations of methacholine hydrochloride in isotonic saline were inhaled, starting at a concentration of 49 $\mu g\ ml^{-1}$ and increasing stepwise to 25 000 $\mu g\ ml^{-1}$. The cumulative dose at the level the reciprocal of the respiratory resistance started to decrease linearly, was determined as the minimum level showing bronchial sensitivity (D_{min}). After 6 days, the patients were readmitted to the hospital, and fibreoptic bronchoscopy and bronchial biopsy were performed. Eight symptoms, i.e. nocturnal wheezing; nocturnal coughing; morning chest tightness; wheezing during the day; limitation of activity; and the effect of exercise, cold air, and dust on asthma symptoms; were evaluated daily using a 4-grade system (3: severe, 2: moderate, 1: mild, 0: none), and the sum of the eight scores was expressed as the 'symptom score' of the day. Symptom score and PEF for the initial 2 weeks were averaged to obtain the initial symptom score and the initial PEF. After-treatment symptom score and PEF were calculated from the records during the final 2 weeks of treatment.

This study is composed of a randomized parallel design without placebo, in which ketotifen, DSCG and BDP preparations were arranged at random and numbered by a third person (pharmacist) in our hospital. Prescriptions were then written out as the order by the doctor (M.H.). In

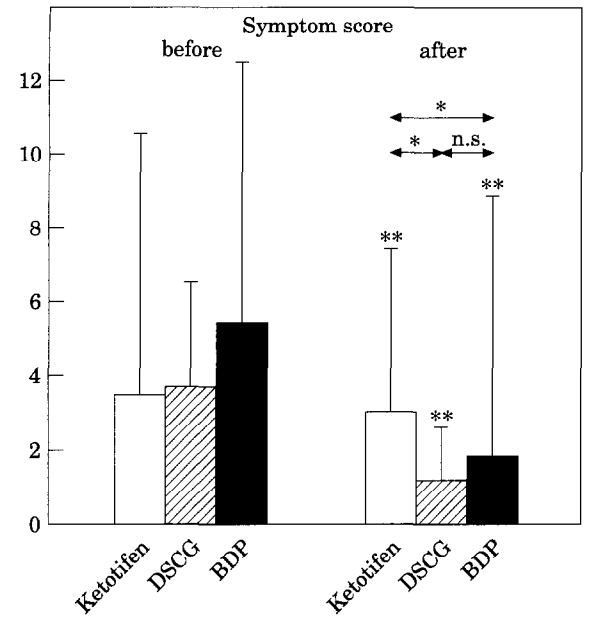

FIG. 1. Mean symptom score in each treatment group. Within-group comparisons were performed with Student's *t*-test (**$P<0.01$). Comparisons of each treatment effects (before-treatment value minus after-treatment value) were analysed by the Bonferroni test (*$P<0.05$).

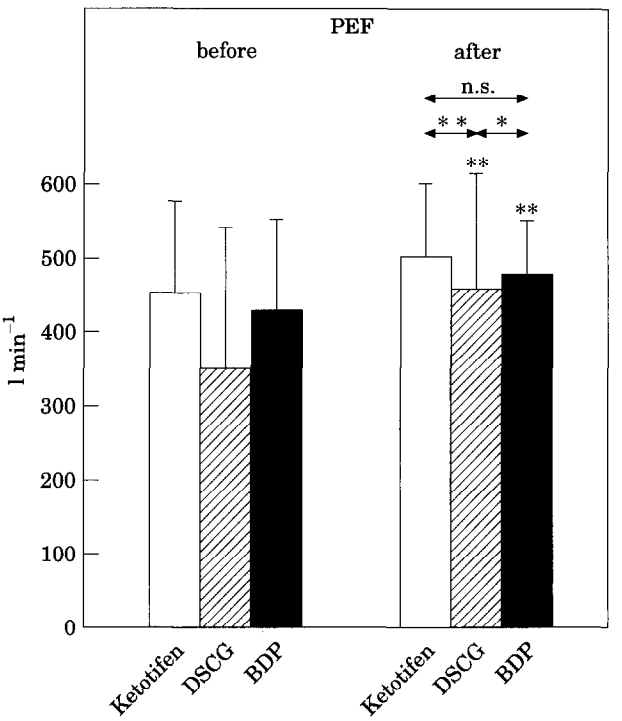

FIG. 2. Mean peak expiratory flow (PEF) in each treatment group. Within-group comparisons were performed with Student's *t*-test (**$P<0.01$). Comparisons of each treatment effects (before-treatment value minus after-treatment value) were analysed by the Bonferroni test (*$P<0.05$, **$P<0.01$).

this way, the patients were randomly allocated to one of the following treatment groups, i.e. oral ketotifen (Sandoz Ltd., Basle, Switzerland) at a dose of 1 mg twice daily, 2 mg q.i.d. DSCG for inhalation (Fujisawa Pharmaceutical Co., Tokyo, Japan), or 100 μg q.i.d. BDP for inhalation (Schering-Plough Pharmaceutical Co., Kenilworth, NJ, U.S.A.), for 3 months. Ketotifen, given in a dose of 2 mg daily for adults and in a dose of 1–2 mg for children, is reported to result in a significant improvement of asthma symptoms (19). The dose of DSCG (8 mg day^{-1}) is equally effective to the inhaled BDP (400 μg day^{-1}) (R. Beasley, pers. comm.). The patients recorded daily use of oral ketotifen, or DSCG or BDP inhalation in their patient diary. Blood test, pulmonary function test, and bronchial responsiveness were conducted in week 11 during treatment, and bronchoscopy and biopsy were conducted during week 12. No patients experienced deterioration of asthma symptoms during the treatment period.

FIBREOPTIC BRONCHOSCOPY

On the day of the bronchoscopy, subjects fasted from 9 a.m. Before the examination, each patient received an i.m. injection of 0·5 mg atropine sulphate and 15 mg pentazocine (Yamanouchi Pharmaceutical Co., Tokyo, Japan). After the throat was anaesthetized with a 4% lidocaine spray, all patients inhaled 200 μg of salbutamol in order to prevent bronchoconstriction. A bronchoscope (BF type 20, Olympus Co., Tokyo, Japan) was inserted through the mouth, and the pharynx, trachea and bronchi were anaesthetized with 2% lidocaine. Oxygen at 4 l min^{-1} was administered via nasal cannulae throughout the procedure. Biopsy forceps (FB-15C, Olympus) were used to collect two or three specimens from the segmental divisions of the right main bronchi. The specimens for immunohistochemistry were mounted in ornithine carbamyl transferase compound (Miles Scientific Naperville, IL, U.S.A.), frozen rapidly in dry ice-acetone, and kept at −70°C until 2 months before sectioning. After bronchoscopy, inhalation of 200 μg salbutamol was administered. In order to assess the degree of the inflammatory cell infiltration into the airways, second biopsy specimens were taken from the same airway sites despite the possibility that they might include scarred or healed tissue. All patients tolerated the procedure well, and no patients experienced bronchoconstriction.

IMMUNOHISTOCHEMISTRY

Frozen sections (4 μm thick) of the bronchial biopsy specimens were cut on a cryostat, placed on albumin-coated slides, air-dried for 30 min, and fixed for 15 min in cold acetone (−20°C). Sections were treated with 0·3% H_2O_2 in methanol for 10 min to block endogenous peroxidase, then for another 30 min with 10% normal porcine serum to suppress non-specific absorption of immunoglobulins, and incubated for 24 h at 4°C with the following primary monoclonal antibodies to identify these cell types: eosinophils [EG2: cleaved form of eosinophil cationic protein (ECP), dilution 1:30; Kabi Pharmacia Diagnostics,

Uppsala, Sweden, No. 10-9196-01], mast cells (AA1: tryptase, dilution 1:50; Dako, Glostrup, Denmark, No. M7052), CD3 (mature T cells: Beckton Dickinson, San Jose, CA, U.S.A. No. 7340), CD4 (helper/inducer T cells: Beckton Dickinson, San Jose, CA, U.S.A. No. 6320), and CD8 (suppressor/cytotoxic T cells: dilution 1:30 each; Beckton Dickinson, San Jose, CA, U.S.A. No. 6310). After being washed with phosphate-buffered saline (PBS), the sections were reacted with peroxidase-labelled anti-mouse immunogloblin G (IgG) (Biosource International, Camarillo, CA, U.S.A.) as the secondary antibody, for 60 min at room temperature, and then washed again with PBS. The peroxidase reaction was developed in 3·3-diaminobenzidine solution (20 mg dl^{-1} in Tris buffer, pH 7·6) containing 0·03% H_2O_2. Finally, the nuclei were counterstained with methylene green and, after being washed in running water, dried with alcohol and treated with xylol. Negative controls were performed by omission of the primary antibodies and by using mouse IgG_1 myeloma proteins as a substitute for the primary antisera.

QUANTIFICATION

To avoid the observer bias, the specimens were all coded prior to analysis by a co-author (J.J.S.) and the blinded slides were examined (by Y.N.) microscopically (Olympus) at × 400 magnification. Numbers of positively stained cells were counted in the lamina propria, beneath the epithelial basement membrane, in an area excluding mucosal glands, bronchial smooth muscle and vessels. An interactive video display system (Olympus) and software for two-dimensional image analysis (NEC Co., Tokyo, Japan) were used to compute the area of the lamina propria in each section. The results were expressed as the number of each cell type per square millimetre of the lamina propria.

STATISTICS

The results for clinical data were normally distributed, and paired data were analysed using the two-tailed paired Student's *t*-test. For multiple correlation, Bonferoni's correlation was used to examine the differences in the before-treatment value minus the after-treatment value between the groups. Because the data for cell counts in the bronchial mucosa showed a positive skew distribution, non-parametric statistical tests were conducted. Paired data for the before- and after-treatment cell counts for immunohistochemistry were analysed by Wilcoxon's matched-pair signed rank test. The effects of each drug were computed by the differences in the before-treatment value minus the after-treatment value, and the group differences in the effects were analysed with the Kruskal–Wallis test. Data were expressed as median and range. A P value of $<0·05$ was regarded as statistically significant. The coefficients of variance for repeated counting by one observer for the immunostaining were 7% for EG2, 6% for AA1, 12% for CD3, 9% for CD4, and 7% for CD8.

Results

Randomization resulted in 13 patients receiving ketotifen, nine patients receiving DSCG, and 10 patients receiving BDP. Their characteristics are summarized in Table 1.

CLINICAL DATA

Symptom score after treatment in each group decreased significantly ($P<0·01$, Fig. 1), and a significant difference after treatment was observed between the ketotifen group and the DSCG group or BDP group ($P<0·05$ each, Fig. 1). PEF increased significantly after treatment in the DSCG and BDP groups ($P<0·01$, Fig. 2; Table 2), but not in the ketotifen group; and significant difference after treatment was observed between the ketotifen group and DSCG group ($P<0·05$). FEV_1% increased after treatment in the DSCG and BDP groups ($P<0·05$, Fig. 3; Table 2), but not in the ketotifen group; and significant difference after treatment was observed between the ketotifen group and DSCG group ($P<0·01$) and BDP ($P<0·05$) group. Bronchial responsiveness in terms of D_{min} in each group increased significantly after treatment ($P<0·01$, Fig. 4; Table 2); however, after treatment, there were no significant group differences. There were also no significant changes in allergen – specific IgE or total serum IgE level, before and after treatment (data not shown).

IMMUNOHISTOCHEMISTRY

At baseline, there were no significant differences in bronchial mucosal cells among the three groups. $EG2^+$ eosinophils in each group decreased significantly after treatment ($P<0·01$ each, Fig. 5) and the mean cell counts before and after treatment, and their ranges, are shown in Table 3. $AA1^+$ mast cell counts significantly decreased after treatment in the DSCG ($P<0·05$) and BDP ($P<0·01$) groups, but not in the ketotifen group (Fig. 6, Table 3). $CD3^+$ T cells in each group decreased significantly after treatment ($P<0·01$ each, Fig. 7; Table 3). $CD4^+$ T cells in each group also decreased significantly ($P<0·01$ each, Fig. 8; Table 3). $CD8^+$ T cells after treatment decreased only in the DSCG group ($P<0·01$ each, Fig. 9; Table 3). After treatment, only the $CD8^+$ T cell count was significantly different between the ketotifen group and DSCG group, and between the DSCG group and BDP group (Fig. 9).

Discussion

No group of subjects receiving a placebo was included because of constraints imposed by our Ethics Committee, who considered a placebo group unjustified. Placebo-controlled studies are rare in Japan, in particular, when the study includes a medication of which the efficacy is being established. Therefore, the results of our study should be interpreted with some caution, and it cannot be concluded that the improvements in inflammation and asthma symptoms are due solely to the effects of the drugs.

TABLE 2. Pulmonary function data before and after treatment

Subject no.	PEF ($l\ min^{-1}$)		FEV_1% pred.		D_{min} (unit)	
	Before	After	Before	After	Before	After
Ketotifen						
1	480	500	89·7	90·0	2·24	3·06
2	550	580	60·3	53·3	0·34	0·28
3	580	600	82·9	83·4	0·26	1·0
4	500	480	64·6	64·7	1·74	4·95
5	480	500	82·1	85·0	0·07	0·22
6	350	350	64·2	65·6	0·16	0·23
7	400	510	84·4	84·4	0·15	0·17
8	320	450	66·2	73·3	0·39	0·6
9	450	400	65·0	63·0	0·32	0·3
10	420	490	72·0	80·0	0·2	0·45
11	450	500	75·8	86·6	0·99	1·73
12	350	370	89·9	91·3	0·56	2·6
13	450	500	58·7	71·8	0·11	0·34
Median	450	500	72·0	80·0	0·32	0·45**
DSCG						
1	370	480	90·5	93·5	2·56	4·1
2	480	630	60·7	67·7	0·34	0·73
3	300	400	61·8	70·4	1·25	2·1
4	370	450	64·4	68·3	0·32	0·56
5	300	380	63·2	72·5	0·52	1·26
6	320	400	57·6	64·9	0·21	0·84
7	350	420	61·9	74·6	0·15	0·47
8	540	600	63·5	76·8	0·33	0·73
9	340	580	65·1	83·6	0·64	0·73
Median	350	450**	63·2	72·5*	0·34	0·73**
BDP						
1	480	500	70·2	75·5	2·03	3·07
2	510	550	65·0	70·7	0·73	2·06
3	330	420	70·5	76·9	1·41	1·56
4	330	330	70·3	80·4	0·39	0·55
5	550	570	63·0	73·0	0·63	1·41
6	380	450	74·9	79·9	0·29	0·55
7	250	350	59·5	65·1	0·35	0·46
8	490	540	59·4	70·5	0·21	1·88
9	450	520	67·0	78·0	1·2	2·4
10	400	420	65·0	72·0	0·92	1·7
Median	425	475**	66·0	74·3*	0·63	1·63**

*$P<0·05$, **$P<0·01$: for comparison between before and after treatment.

Before and during the study, all patients received β_2-agonist inhalation when necessary. Laitinen *et al.* (15) compared budesonide, terbutaline and placebo, and found that in the group treated with regular terbutaline, only the number of lymphocytes decreased significantly. In a study similar to ours, Djukanović *et al.* (17) demonstrated that BDP possesses anti-inflammatory effects on the bronchial mucosa and positive effects on asthma symptoms and bronchial responsiveness. The drugs used in treatment for asthma can be divided into two groups: the group of drugs which possess anti-inflammatory effects and can prevent asthma attacks (control drugs), and the other group of drugs which act quickly against bronchoconstriction (reliever drugs). According to our findings, ketotifen, DSCG, and BDP are shown to belong to the 'control drugs' group.

Ketotifen significantly reduced the number of activated eosinophils in the bronchial mucosa of patients with asthma. Inhibition of eosinophil aggregation in the airway caused by platelet-activating factor (20) and cytokines (21) has been reported. The present study demonstrated the anti-inflammatory effect of ketotifen, in human bronchial mucosa *in vivo*. In a study (22) examining the eosinophil content of the sputum of asthmatics with and without Intal

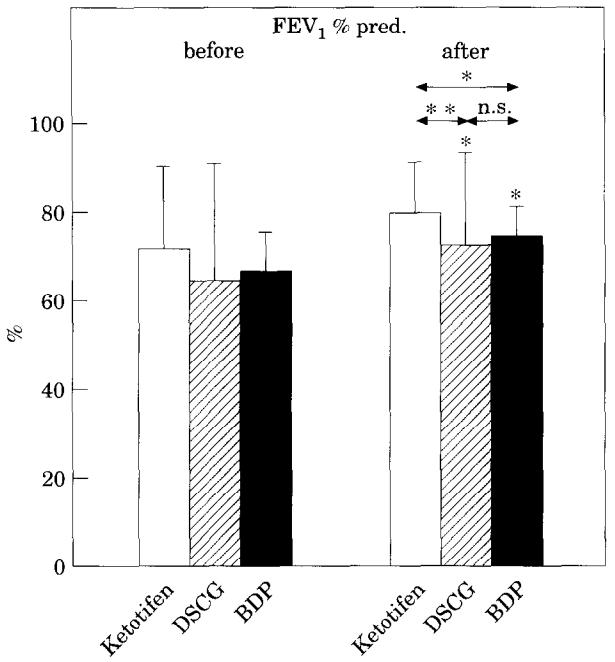

FIG. 3. Mean FEV_1% pred. in each treatment group. Within-group comparisons were performed with Student's *t*-test (*$P<0.05$). Comparisons of each treatment effects (before-treatment value minus after-treatment value) were analysed by the Bonferroni test (*$P<0.05$, **$P<0.01$).

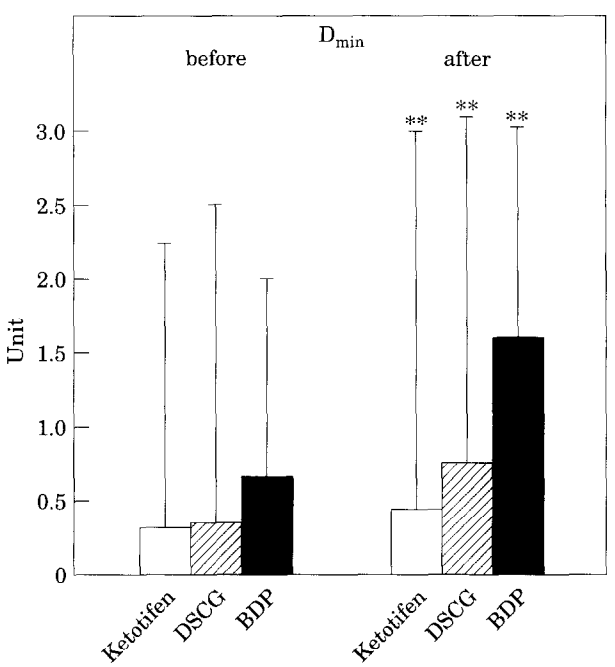

FIG. 4. Bronchial responsiveness to methacholine (D_{min}) in each treatment group. Within-group comparisons were performed with Student's *t*-test (**$P<0.01$).

treatment, the greatest response to Intal was observed in patients who had numerous eosinophils in the sputum. Reduction in eosinophil numbers in BAL after DSCG inhalation was also reported by Diaz *et al.* (23). Our

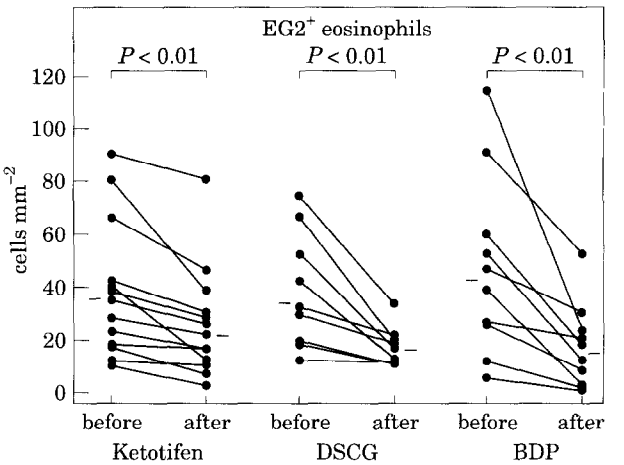

FIG. 5. $EG2^+$ eosinophil counts in each patient, expressed as numbers per square millimetre of lamina propria of bronchial mucosa. The short horizontal bars represent median values.

findings demonstrated that DSCG inhibits eosinophil infiltration in the bronchial mucosa. The number of $EG2^+$ eosinophils in the bronchial mucosa significantly decreased after BDP treatment. This is consistent with the contention that steroid inhalation makes eosinophil cationic protein (ECP) levels in BAL decrease (24), and it is consistent with the reports that the number of eosinophils in the bronchial mucosa decreases after steroid inhalation (16,17).

Several studies have suggested that there is no difference in the number of mast cells between asthma patients and healthy subjects (4,25). Conversely, some other studies have indicated that the number of mast cells increases in the epithelium of asthmatic patients (26) and in BAL (27,28). Though there may be some association of mast cell numbers with the fragility of the epithelium, this increase probably reflects the recruitment of mast cells from the deeper layers to the epithelium. Compared with the ketotifen group, $AA1^+$ mast cell counts in the DSCG and BDP groups significantly decreased. The lack of the effect of ketotifen on the number of mast cells would be attributable to the fact that mast cells were counted only in the submucosal area, but not in the epithelium, in order to exclude the effect of biopsy injury on the epithelium. We hypothesize that DSCG and BDP possess stronger anti-inflammatory effect than ketotifen.

Asthma patients are reported to have a large number of T cells in the airway epithelium and in the lamina propria, than normal subjects (29). Robinson *et al.* (30) analysed cytokine mRNA in the cells obtained by BAL by *in situ* hybridization, and showed that various types of cells produce IL-4 or IL-5. Recently, ketotifen was reported to be effective against murine recombinant IL-5-induced airway inflammation in guinea-pigs (31). We assumed that the suppressive effect of ketotifen on cytokine (IL-4 or IL-5) production results in a significant decrease of $CD3^+$ and $CD4^+$ T cells, but does not affect the number of $CD8^+$ T cells. Further research is necessary to clarify this point. In the present study, all the cell counts after DSCG treatment

TABLE 3. Effects of treatment on cell counts in the bronchial mucosa

	EG2		AA1		CD3		CD4		CD8	
	Before	After	Before	After	Before	After	Before	After	Before	After
Ketotifen	35	22**	22	21·5	120	84**	75	43**	50	51
	(10–90)	(2–80)	(12–54)	(8–55)	(80–200)	(47–110)	(55–120)	(28–78)	(41–81)	(38–70)
DSCG	32	16**	24	16*	115	89**	81	44**	52	36**
	(12–74)	(10–33)	(16–64)	(13–24)	(74–180)	(56–128)	(43–140)	(36–70)	(37–87)	(28–52)
BDP	42	15**	24	18·5**	140	90**	98	46·5**	67	60·5
	(5–113)	(0–52)	(12–41)	(9–22)	(64–300)	(35–148)	(36–198)	(15–90)	(25–106)	(13–100)

Values are median (range). *$P<0.05$, **$P<0.01$: for comparison between before and after treatment.

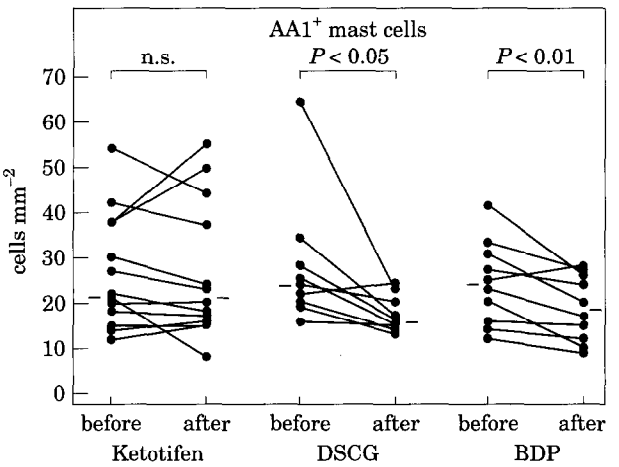

FIG. 6. $AA1^+$ mast cell counts in each patient, expressed as numbers per square millimetre of lamina propria of bronchial mucosa. The short horizontal bars represent median values.

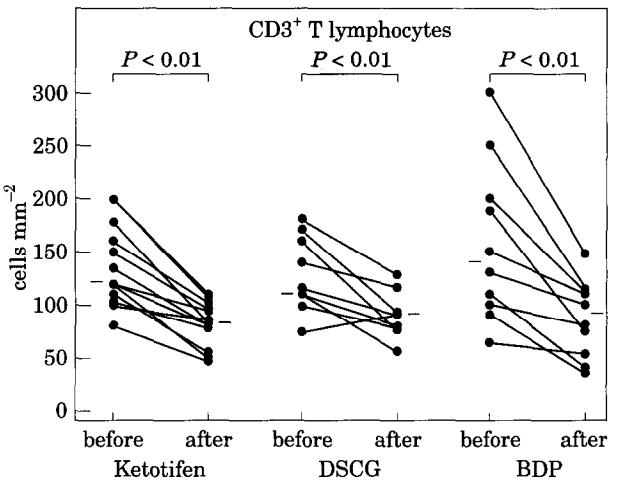

FIG. 7. $CD3^+$ T-lymphocyte counts in each patient, expressed as numbers per square millimetre of lamina propria of bronchial mucosa. The short horizontal bars represent median values.

became significantly lower. The exact mechanisms of action of DSCG are not fully understood, although it is known to have cell- and mediator-selective suppressive effects on the other inflammatory cells (e.g. eosinophils, monocytes, macrophages) (32). $CD3^+$ and $CD4^+$ T cells decreased significantly after BDP inhalation, but $CD8^+$ T cells did not. A possible reason is that steroids may decrease the number of T cells (33), in particular, that of $CD4^+$ cells (34), in peripheral blood. Bentley *et al.* (35) reported that the number of cells which express IL-4 and IL-5 mRNAs in the bronchial mucosa of asthmatic patients, decreased significantly after steroid therapy. The reduction in the number of T cells after BDP inhalation in our study may be a result of the inhibited cytokine production caused by BDP. Recently, it has been suggested that each group in such a biopsy study as ours needs at least 15 patients in order to provide sufficient statistical power to detect most of the changes in the biopsies of the airways (36). In the present study, each group had a limited number of patients, i.e. between nine and 13 subjects, and no significant changes were observed in the counts of eosinophils, mast cells, and $CD4^+$ T cells in spite of the use of BDP which possesses the most potent anti-inflammatory effect. If an examination with a larger number of subjects is conducted, it could demonstrate additional effects of the drugs on inflammatory cells.

Our post-treatment clinical data showed significant improvements in the symptom score and bronchial responsiveness in the three groups. The change in PEF was the largest in the DSCG group. Both the DSCG and BDP groups showed larger changes in FEV_1% than the ketotifen group. In long-term clinical trials, the efficacy and tolerance of ketotifen have been reported (37,38), and ketotifen has been reported to improve pulmonary function (12,38,39), though contradictory data (11,19) have also been published. DSCG reduces severity of asthma symptoms, frequency of exacerbations (40,41), and airway responsiveness, in patients with asthma (42). Other studies also demonstrated that ketotifen, DSCG and BDP improve lung function, decrease airway hyperresponsiveness, reduce the severity of symptoms, decrease frequency of exacerbations, and improve quality of life (43–45).

In the present study, ketotifen, DSCG, and BDP all exerted anti-inflammatory activity in the bronchial mucosa of asthmatic patients, and the DSCG and BDP groups demonstrated superior clinical responses

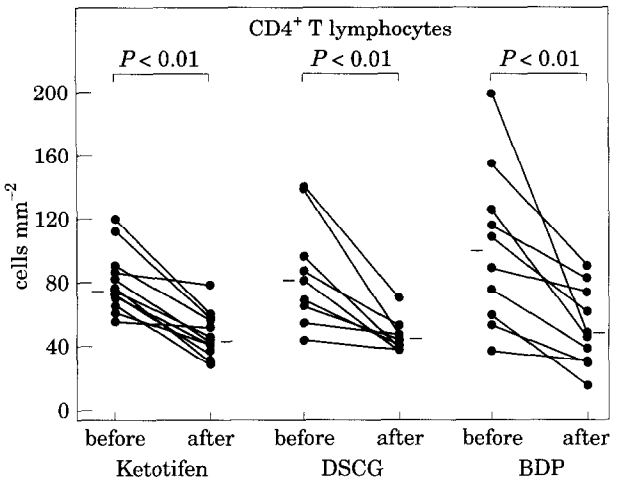

FIG. 8. $CD4^+$ T-lymphocytes counts in each patient, expressed as numbers per square millimetre of lamina propria of bronchial mucosa. The short horizontal bars represent median values.

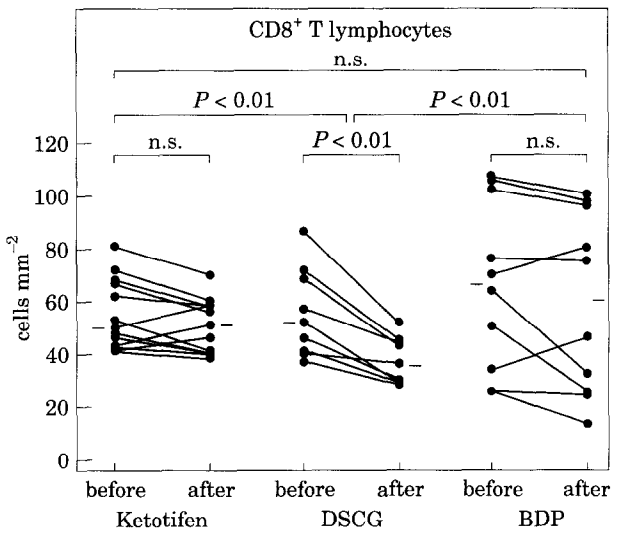

FIG. 9. $CD8^+$ T-lymphocytes counts in each patient, expressed as numbers per square millimetre of lamina propria of bronchial mucosa. The short horizontal bars represent median values. The differences (before-treatment value minus after-treatment value) in cell counts for each treatment group were compared by using the Kruskal–Wallis test.

as compared to the ketotifen group. We conclude that DSCG and BDP could result in a better improvement than ketotifen.

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